510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k042644

B. Purpose for Submission:
New Devices

C. Measurand:
Anti-Tissue Transglutaminase IgA and IgG

D. Type of Test:
Qualitative and Semi-quantitative ELISA

E. Applicant:
AESKU, Inc.

F. Proprietary and Established Names:
AESKULISA® tTg A Protocol 30-15-15 REF 7503
AESKULISA® tTg A Protocol 30-30-30 REF 30-7503
AESKULISA® tTg G Protocol 30-15-15 REF 7504
AESKULISA® tTg G Protocol 30-30-30 REF 30-7504

G. Regulatory Information:
1. Regulation section:
   21 CFR 866.5660, Multiple autoantibodies immunological test system
2. Classification:
   II
3. Product code:
   MVM, Autoantibodies, Endomysial (Tissue Transglutaminase)
4. Panel:
   Immunology 82

H. Intended Use:
1. Intended use(s):
   The AESKULISA® tTg A is a solid phase enzyme immunoassay for the semiquantitative and qualitative detection of IgA antibodies against tissue transglutaminase (tTG) in human serum. The assay is an aid in the diagnosis of celiac disease (gluten-sensitive enteropathy) and should be used in conjunction with other serological tests and clinical findings. For *in vitro* diagnostic use only.

   The AESKULISA® tTg G is a solid phase enzyme immunoassay for the semiquantitative and qualitative detection of IgG antibodies against tissue transglutaminase (tTG) in human serum. The assay is an aid in the diagnosis of

celiac disease (gluten-sensitive enteropathy) and should be used in conjunction with other serological tests and clinical findings. For *in vitro* diagnostic use only.

2. Indication(s) for use:
   Same as intended use.

3. Special conditions for use statement(s):
   For prescription use only.

4. Special instrument requirements:
   Microplate reader capable of measuring OD at 450 nm.
   Microplate washing device (300µL repeating or multichannel pipette or automated system).

I. Device Description:

Each device contains the following: microplate strips with breakaway microwells coated with recombinant human tTG and Gliadin-specific peptide antigen, six levels of calibrators (0, 3, 10, 30, 100, 300 U/mL); positive, negative and cut-off controls (human serum, diluted); wash buffer concentrate; sample buffer; goat anti-human immunoglobulin (IgG or IgA) horseradish peroxidase conjugate; 3,3',5,5' tetramethylbenzidine (TMB)/H_2O_2 substrate; and 1M hydrochloric acid stop solution.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   ImmuLisa™ Anti-Human Tissue Transglutaminase (hu-tTG) Antibody IgA ELISA and ImmuLisa™ Anti-Human Tissue Transglutaminase (hu-tTG) Antibody IgG ELISA

2. Predicate 510(k) number(s):
   K032571 and K040095

3. Comparison with predicate:

Similarities		
Item	New Device	Predicate Device
AESKULISA® tTg G: Intended use	To aid in the diagnosis of celiac disease.	To aid in the diagnosis of celiac disease.
Technology	ELISA	Same
Assay Format	Qualitative and Semi-quantitative	Same
Positive and negative controls	Ready to use	Same
Stop solution	Ready to use	Same
Platform	96 well microtiter plates	Same

Differences		
Item	Device	Predicate
AESKULISA® tTg A: Intended use	To aid in the diagnosis of celiac disease.	To aid in the diagnosis of IgA deficient patients with celiac disease in patients with IgA deficiency.
Antigen	Recombinant human tissue-Transglutaminase and Gliadin-specific peptides	Human tTG
Calibrators	6 levels: 0, 3, 10, 30, 100, 300 U/mL	4 levels: 19, 42, 68, 106 U/mL
Sample type and dilution	Serum at 1:101	Serum at 1:51
Sample volume required	100 µL	10 µL
Enzyme-Conjugate	Horseradish Peroxidase	Alkaline phosphatase
Sample buffer/diluent	5x concentrated	Ready to use
Wash buffer	50x concentrated	Powder
Substrate	TMB/H2O2	pNPP
Incubation times	tTg-A and tTg-G (REF 7503 and REF 7504 respectively) Protocol A: 30-15-15 minutes tTg-A and tTg-G (REF 30-7503 and REF 30-7504 respectively) Protocol B: 30-30-30 minutes	30-30-30 minutes
OD reading	450 nm	405 nm
Cut-off	15 U/mL	20 U/mL

K. Standard/Guidance Document Referenced (if applicable):

None referenced.

L. Test Principle:

The AESKULISA® tTg A and the AESKULISA® tTg G devices are solid phase enzyme immunoassays for the semiquantitative and qualitative detection of IgA and IgG antibodies respectively, against tTG in human serum. The wells of a microplate are coated with recombinant human tTG and Gliadin-specific peptide antigen. Antibodies specific to tTG present in the patient sample bind to the antigen. In a second step, the enzyme labeled second antibody (conjugate) of specific isotype (IgA or IgG) binds to the antigen-antibody complex which leads to the formation of an enzyme labeled conjugate-antibody-antigen complex. The enzyme-labeled antigen-antibody complex converts the added substrate to form a colored solution. The rate of color formation from the chromogen is a function of the amount of conjugate

complexed with the bound antibody and is proportional to the initial concentration of the respective antibodies in the patient serum. The results are read spectrophotometrically and are interpreted by comparison to a cut-off calibrator (qualitative) or a standard curve (semi-quantitative).

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. *Precision/Reproducibility*:
   Three samples (high, medium, near the cut-off) were assayed 18 times on three microplates of the specific antibody isotype, on different days for the inter-assay study. The same three samples were assayed 18 times on one microplate of the specific antibody type for the intra-assay study. Both studies were performed on Protocol A with the 30-15-15 incubation times. Target values for the studies were set at variance ≤ 10%. The intra-assay variation range for Anti-tTg A was from 5.2% to 7.0% and for Anti-tTg G was from 3.1% to 4.6%. The inter-assay variation range for Anti-tTg A was from 0.8% to 4.2% and for Anti-tTg G was 4.3% to 9.1%. All the ranges were within the target values.

	Intra-assay Variation		
Anti-tTg A	Sample 1	Sample 2	Sample 3
CV (%)	7.0	5.2	5.5
Mean (U/mL)	13.8	56.7	166.5
Anti-tTg G			
CV (%)	3.1	3.5	4.6
Mean (U/mL)	19.2	100.8	152.8

	Inter-assay Variation		
Anti-tTg A	Sample 1	Sample 2	Sample 3
CV (%)	2.3	0.8	4.2
Mean U/mL)	10.1	38.9	169.4
Anti-tTg G			
CV (%)	4.3	7.6	9.1
Mean (U/mL)	16.7	97.4	194.4

   b. *Linearity/assay reportable range:*
      i. Study design: Four samples known to contain different levels of Anti-tTg IgA and another four samples known to contain different levels of Anti-tTg IgG were chosen and serially diluted to determine the linearity of the assay. From an initial dilution of 1/100, further dilutions of 1:200, 1:400 and 1:800 were made.

      ii. Results/Acceptance criteria: The Anti-tTg A assay had a recovery range of 71.9% to 106.6%. The Anti-tTg G had a recovery range of 78.8% to 109.7%. Based on this study, the dynamic range of the assay is 1 to 100 U/mL.

   c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

The standards are prepared in-house and arbitrary units are assigned during the development process. The positive and negative controls are prepared in house.

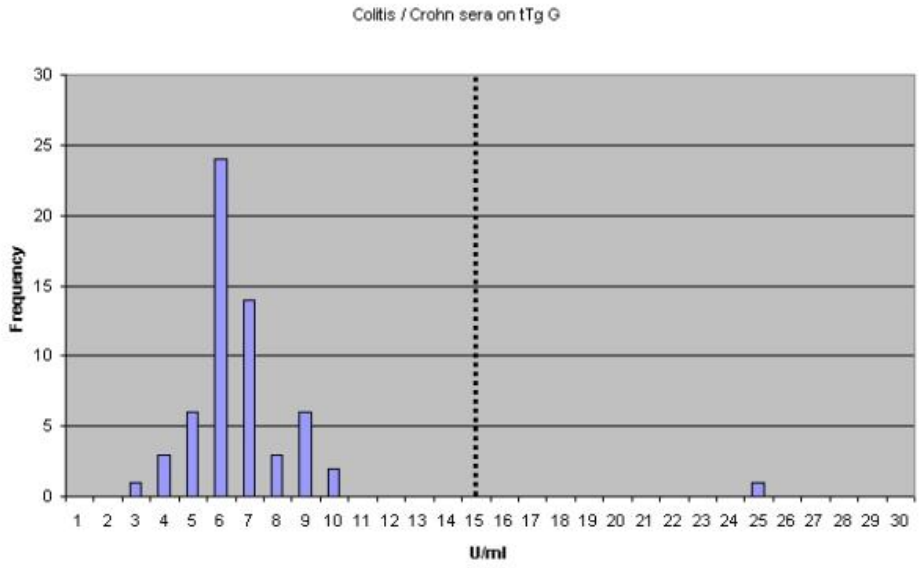
d. Detection limit:

The sample diluent was diluted according to the directions for use and measured 32 times for each assay. The value for the analytical sensitivity (detection limit) was calculated as the mean of the optical densities of the sample diluent. The analytical sensitivity was 1.0 U/mL.

e. Analytical specificity:

i. Interference by endogenous substances: No data provided. The package insert states that icteric, lipemic, hemolyzed or bacterially contaminated samples should not be used in these assays.

ii. Crossreactivity to other autoantibodies: The Anti-tTg A and Anti-tTg G assays were tested with sixty sera from patients with autoimmune diseases like Crohn's disease (58) and ulcerative colitis (2). Both assays have a cut-off value of 15 U/mL. The results with the Anti-tTg A assay were all below 15 U/mL. The results with the Anti-tTg G assay had 59 samples below 15 U/mL and 1 sample (with ulcerative colitis) was above 15 U/mL. The detailed results are depicted in the two graphs below.



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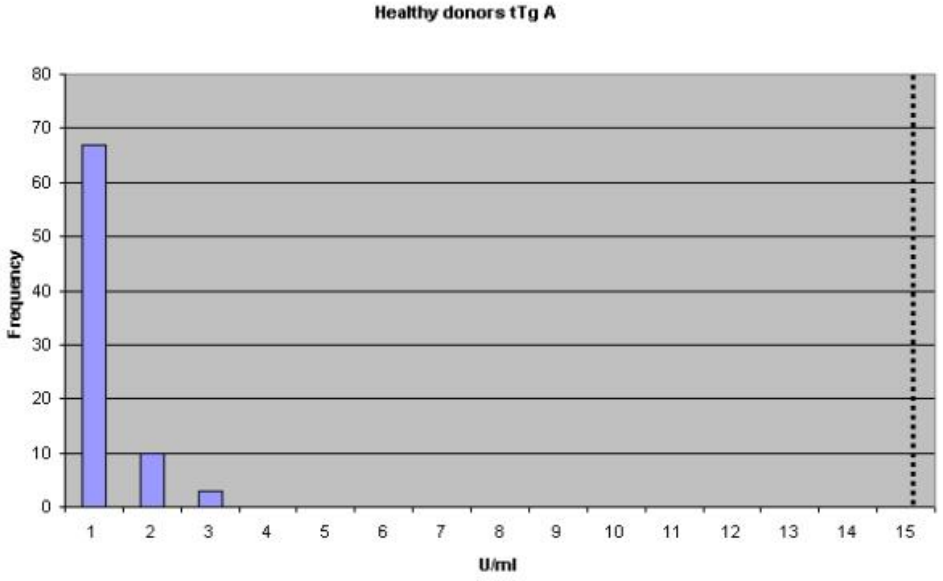
iii. Crossreactivity with Gliadin positive sera: Seven Gliadin positive Celiac disease sera were tested with the two assays to ensure that the Gliadin specific peptides which are crosslinked to the tTg in the device, do not cross react with Gliadin positive sera. All seven samples had results below the assay cut-off.

f. Assay cut-off:

The cut-off value of 15 U/mL for the Anti-tTg A and Anti-tTg G devices was

based on the testing of 80 healthy donor samples. 100% and 97.5% of the 80 subjects were negative for the anti-tTg A assay and the anti-tTg G assay respectively. The results are depicted in the two graphs below. The Anti-tTg A graph shows the 80 subjects had results below 3 U/mL. The Anti-tTg G graph shows the 77 subjects had results below 8 U/mL, and the three subjects above the cut-off had results of 15, 17 and 21 U/mL.



2. Comparison studies:
   a. Method comparison with predicate device:
      i. Anti-tTg A assay – Testing was performed on 165 samples which included 64 confirmed Non-treated Celiac Disease (CD), 38 Celiac Disease Gluten-Free Diet (CD GFD), 51 Crohn's Disease (MC), 2 Lactose intolerance, 4 Helminthiasis, 2 Ulcerative Colitis (UC) and 4 healthy donor. Twenty-seven percent (27%) of the sera were from younger than 26 years, 41% were between 26 - 46 years, and 32% were from older than 46 years of age. Fifty-nine percent of the sera were from females and 41% were from males. The samples were tested on the Anti-tTg A assay Protocol A (REF 7503) with incubation period of 30-15-15 minutes. Analysis was performed according to the instructions for use.

Anti-tTg A Comparison Data:

		IMMULISA tTg-A (predicate)		
		positive	negative	Total
AESKULISA tTg-A	positive	25	42	67
	negative	2	96	98
	Total	27	138	165

Positive percent agreement 92.6% (25/27)
Negative percent agreement 69.6% (96/138)
Overall percent Agreement 73.3% (121/165)

ii. Anti-tTg G assay – Testing was performed on 185 samples which included the 165 samples for the Anti-tTg A assay. The additional 20 samples were IgA Deficient Celiac Disease sera. These 185 samples were tested on the Anti-tTg G assay Protocol A (REF 7503) with incubation period of 30-15-15 minutes. Analysis was performed according to the instructions for use. The results were as follows:

Anti-tTg G Comparison Data:

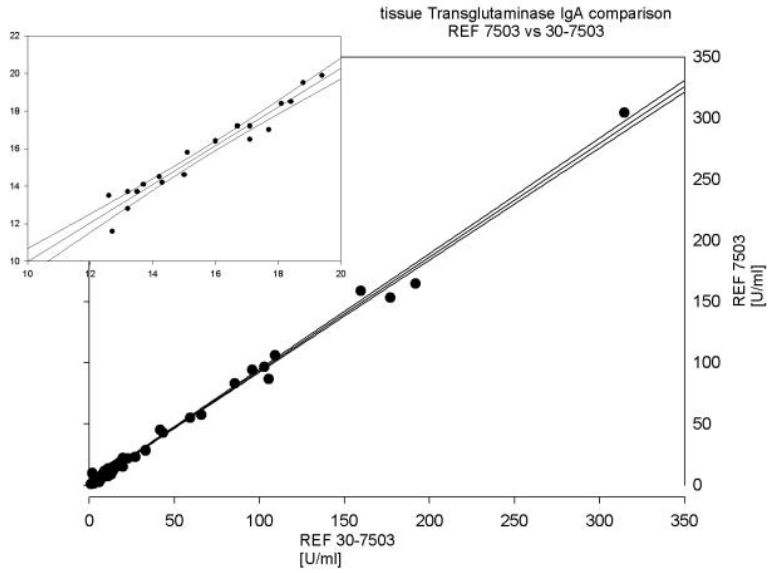
		IMMULISA tTg-G (predicate)		
		positive	negative	total
AESKULISA tTg-G	positive	18	67	85
	negative	4	96	100
	Total	22	163	185

Positive percent agreement 81.8 % (18/22)
Negative percent agreement 58.9 % (96/163)
Overall percent agreement 61.6 % (114/185)

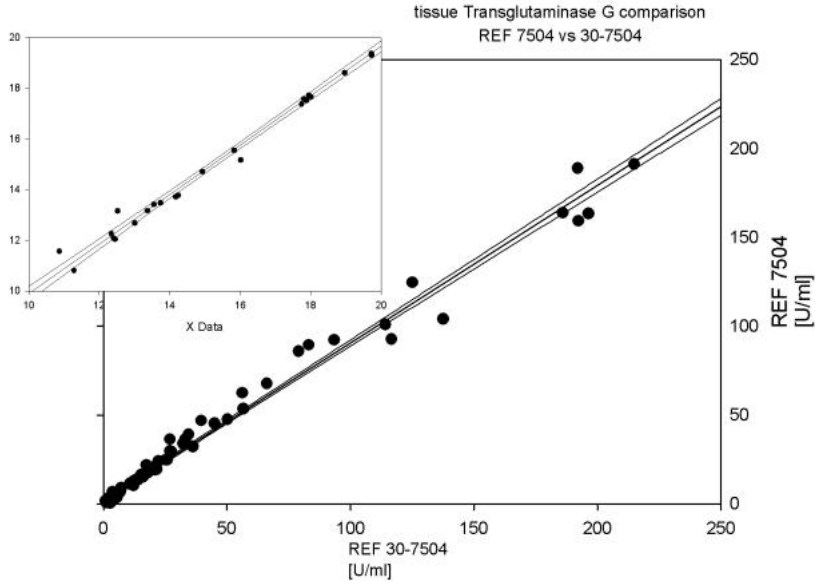
Of the 20 IgA deficient Celiac disease patient sera, both devices gave negative results on two sera and positive results on nine sera. The new device detected 9 more positive sera than the predicate device.

The discrepancies observed between the predicate and the new device could be due to the antigens used for capturing the autoantibodies. The antigen of the new device is a recombinant human tissue-Transglutaminase and Gliadin-specific peptides, while the predicate device antigen is tissue-Transglutaminase purified from human tissue.

iii. Protocol A and Protocol B comparison studies:
The comparability of the Anti-tTg A data were assessed with 76 sera tested on both REF 7503 (30-15-15 minute Protocol A) and REF 30-7503 (30-30-30 minute Protocol B). The linear regression analysis is depicted in the large figure below with $r^2 = 0.99$. The upper left small figure shows selected results close to the 15 U/mL cut-off range with $r^2 = 0.95$.



The comparability of the Anti-tTg G data were assessed with 75 sera tested on both REF 7503 (30-15-15 minute Protocol A) and REF 30-7503 (30-30-30 minute Protocol B). The linear regression analysis is depicted in the large figure below with $r^2 = 0.99$. The upper left small figure shows selected results close to the 15 U/mL cut-off range with $r^2 = 0.99$.



b. Matrix comparison:
Not applicable.

3. Clinical studies:
   a. Clinical Sensitivity and specificity:
   The two tables below show the same samples mentioned in the above comparison data, but the results are according to the Celiac Disease

diagnosis.

Celiac Disease sera with Diagnosis with Anti-tTg A

		Diagnosis		
		Positive	Negative	Total
AESKULISA tTg-A	Positive	62	5	67
	Negative	2	96	98
	Total	64	101	165

Sensitivity = 96.9% (62/64)
Specificity = 95.0% (96/101)

Celiac Disease sera with Diagnosis with Anti-tTg G

		Diagnosis		
		Positive	Negative	Total
AESKULISA tTg-G	Positive	80	5	85
	Negative	4	96	100
	Total	84	101	185

Sensitivity = 95.2 % (80/84)
Specificity = 95.0% (96/101)

b. Other clinical supportive data (when a. and b. are not applicable):
Not applicable.

4. Clinical cut-off**:**
Same as assay cut-off.

5. Expected values/Reference range:
Expected values in the normal population should be negative.

N. Proposed Labeling:
The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:
The submitted information in this premarket notification is complete and supports a substantial equivalence decision.